Supplementary Discussion

An Orc3 insertion creates a binding platform for Orc6

Of the structural variations present in the Orc1-5 AAA+ folds, the domain insertion in Orc3 is known to play an important role in the assembly of the ORC hexamer. Most if not all eukaryotic Orc3 homologs contain the insertion, which in yeast, fly and human ORC, serves as a critical binding site for Orc6[14]. In the structure, the Orc3 insertion forms a bi-lobed, α-helical domain that extends from the principal ORC body (**Extended Data Fig. 4a, b**); computational searches of the structural database indicate that this region constitutes a novel fold. Most of the surface-exposed residues of the Orc3 insertion are highly variable, with the exception of two clusters, one that packs against the lid of the Orc3 AAA+ domain and another that binds to Orc6 (**Extended Data Fig. 4c**).

The Orc3•Orc6 interface is itself formed by three helices in Orc3 and a single helix in the C-terminal domain of Orc6 (the register for the build of this region was verified by binding and site-specific crosslinking experiments, **Extended Data Fig. 4b, d-h**). The three Orc3 helices form a triangular, primarily hydrophobic binding pocket for the docking of highly conserved Orc6 amino acids, in particular Tyr225 and Trp228. In agreement with an important role for these residues in Orc3•Orc6 interactions, mutation of Tyr225 or Trp228/Lys229 abrogates Orc6 binding to Orc3 and its recruitment into ORC[14] (**Extended Data Fig. 4e, f**). Notably, Tyr225 in *Drosophila* Orc6 corresponds to Tyr232 in human Orc6, which is mutated to serine in a subset of patients with Meier-

Gorlin syndrome, a primordial dwarfism disorder[69]. Structural modeling indicates that the shorter side chain of serine would not be capable of supporting the local bonding and packing interactions formed by the wild-type tyrosine residue, a defect that likely accounts, at least in part, for the reduced affinity seen between ORC1-5 and mutant Orc6 proteins bearing the Meier-Gorlin syndrome substitution[14].

Supplementary References

14 Bleichert, F. *et al.* A Meier-Gorlin syndrome mutation in a conserved C-terminal helix of Orc6 impedes origin recognition complex formation. *Elife* **2**, e00882, (2013)

69 Bicknell, L. S. *et al.* Mutations in the pre-replication complex cause Meier-Gorlin syndrome. *Nat Genet* **43**, 356-359, (2011)